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Atrial natriuretic peptide and furosemide effects on hydraulic pressure in the renal papilla

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Atrial natriuretic peptide and furosemide effects on hydraulic pressure in the renal papilla. Atrial peptides (ANP) have been shown to preferentially increase blood flow to juxtamedullary nephrons and to augment vasa recta blood flow. To determine the effect of this alteration in intrarenal blood flow distribution on pressure relationships in inner medullary structures and their significance as a determinant of ANP-induced natriuresis, we measured hydraulic pressures in vascular and tubule elements of the renal papilla exposed in Munich-Wistar rats in vivo during an euvolemic baseline period and again during an experimental period. Rats in Group 1 received intravenous infusion of rANP [4-27], administered as a 4 μg/kg prime and 0.5 μg/kg/min continuous infusion, and were maintained euvolemic by plasma replacement. Infusion of ANP resulted in significant natriuresis, diuresis and increase in inulin clearance. Within 90 seconds of initiation of this systemic infusion, vasa recta hydraulic pressures were markedly increased and exceeded the small pressure increment occurring in loops of Henle and collecting ducts. Infusion of furosemide in Group 2 rats at a dosage which reproduced the increase in urine flow in Group 1 was associated with small and equivalent increases in both vascular and tubule elements, indicating that the differential pressure response observed in Group 1 was not due to increased tubule fluid flow rates, but was rather a specific ANP-induced vascular effect. Group 3 rats received an infusion of ANP in a setting where its whole kidney hemodynamic effects were prevented. This resulted in a marked blunting of natriuresis and diuresis, and obliteration of the pressure differential between vasa recta and tubules observed in Group 1. Thus, the hydraulic pressure imbalance between papillary vascular and tubule structures occurring during infusion of ANP in euvolemic Group 1 rats may impede fluid reabsorption from local papillary interstitium, and thereby serve as an important mechanism of ANP-induced natriuresis and diuresis.

Several mechanisms have been suggested to contribute to the augmentation of renal sodium excretion occurring in response to infusions of atrial natriuretic peptides (ANP). These have included an increase in the glomerular filtration rate (GFR) [1, 2], an alteration of intrarenal blood flow distribution toward juxtamedullary nephrons with attendant "medullary washout" [3, 4], and direct inhibitory effects on tubule sodium transport in proximal [5, 6] and terminal nephron segments [7, 8].

The relative importance of these diverse ANP effects remains unclear. It is evident, however, that renal hemodynamic actions of ANP are important determinants of the natriuretic response to ANP infusions. Lowering of renal perfusion pressure by pre-renal arterial clamping prior to, or during, ANP administration has been shown to markedly blunt or abolish the natriuresis [1, 9, 4]. Since clamping also prevented an ANP-induced rise in GFR, the enhancement of GFR by ANP was felt to be a primary factor responsible for its natriuretic effect [1]. However, several studies have demonstrated increases in renal sodium excretion in the absence of discernable changes in whole kidney hemodynamics [6, 7, 10]. On the other hand, an additional hemodynamic mechanism which might be operative lies in the renal vasodilatory properties of ANP, in particular its ability to increase vasa recta blood flow [11, 10]. Such actions could give rise to alterations in medullary vasa recta Starling forces, which in turn could influence sodium excretion by altering capillary uptake of reabsorbate in this region.

The present studies were therefore undertaken to characterize the inner medullary hemodynamic response to infusion of ANP. This response was compared to infusion of a direct inhibitor of epithelial sodium transport, furosemide, and to infusion of ANP in a setting where its hemodynamic effects were suppressed.

Methods

Three groups of male Munich-Wistar rats (Simonson Laboratory, Gilroy, California, USA) weighing 89 to 122 g were employed in these studies. All animals were allowed free access to standard rat chow (Rodent Laboratory Chow #5001, Ralston Purina Co., Richmond, Indiana, USA) and tap water.

Micropuncture studies

Rats were anesthetized with Inactin (100 mg/kg, i.p.; Byk Gulden, Konstanz, FRG) and positioned on a temperature regulated table. Following tracheostomy, a polyethylene catheter (PE-50) was inserted into the left femoral artery for periodic blood sampling and for monitoring of mean arterial pressure ($\overline{AP}$) via an electronic transducer (Model P23Db, Statham Instruments Division, Gould Inc., Oxnard, California, USA) coupled to a direct-writing recorder (Model 7754A, Hewlett-Packard Co., Palo Alto, California, USA). Catheters (PE-50) were also inserted into the left femoral vein for infusion of plasma and into the right and left jugular veins for infusion of inulin/para-aminohippurate (PAH) and experimental agents, respectively. To prevent net loss of plasma volume during preparation for micropuncture, all rats received an intravenous infusion of isoncotic rat plasma equivalent to 1% of body weight

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over 45 minutes, after which the infusion rate was reduced to 1.5 ml/kg/hr. A similar protocol has been shown to maintain hematocrit at pre-anesthesia levels in Munich-Wistar rats of comparable age during micropuncture [12]. All animals also received a continuous infusion of 0.9% NaCl containing 3% inulin and 0.8% PAH at the rate of 0.41 ml/hr. Following midline and left subcostal incisions, the right ureter was cannulated with PE-10 tubing, and the left kidney was suspended on a glass holder and bathed continuously with warmed 0.9% NaCl. The left ureter was carefully dissected to provide full exposure of the renal papilla (1.5 to 2.5 mm), which was transilluminated with a fiber-optic light source and also bathed continuously with warmed 0.9% NaCl.

After allowing 60 minutes for equilibration following the reduction in plasma infusion rate, baseline clearance and micropuncture measurements were begun. Urine from the right kidney was collected at 10 to 20 minute intervals for measurement of urine flow rate ($\dot{V}$) and concentrations of sodium, inulin, and PAH. Femoral arterial blood samples were obtained at the midpoint of each urine collection for determination of inulin and PAH concentrations. Time-averaged hydraulic pressures were measured in one to three descending and ascending vasa recta, loops of Henle, and collecting ducts in each animal at accessible points between base and tip of the exposed renal papilla with a continuously recording, servo-nulling system (Model 3, Instrumentation for Physiology and Medicine, San Diego, California, USA) which employed 2 to 3 μm (outer tip diameter) micropipettes filled with 2.0 M NaCl. In this study the base refers to the portion of the exposed papilla nearest to the kidney whereas the tip refers to the opposite end. Hydraulic output from the servo system was coupled electronically to a second channel of the Hewlett-Packard recorder by means of a pressure transducer (Statham Model P23Db).

Group 1 (N = 6)

Following completion of baseline clearance and micropuncture measurements (30 to 40 min), synthetic rat ANP [4-27] (Peninsula Laboratories, Belmont, California, USA) in 0.9% NaCl was infused intravenously as a 4 μg/kg priming dose (0.07 ml) over a two minute interval, followed by a sustaining i.v. infusion at the rate of 0.5 μg/kg/min (0.58 ml/hr). The plasma infusion rate was increased to 0.01 ml/min concurrently with ANP infusion in an attempt to maintain the euvolemic state. This rate was found in pilot studies to maintain a constant hematocrit during infusion of ANP. Clearance studies during this experimental period were begun three to five minutes after completion of the ANP priming dose. This time interval allowed maximal urine flow rates to be achieved and thereby obviated urinary dead-space artifact during the transition from low to high urine flow rates. Hydraulic pressure measurements in papillary vascular and tubule elements were repeated in random order at the same sites sampled during the control period (for 90% of the determinations), beginning 10 minutes after the initiation of the priming ANP dose and ending approximately 20 minutes later.

Group 2 (N = 7)

These rats were subjected to an identical protocol as detailed for Group 1 except that, instead of ANP, an intravenous infusion of furosemide (Lasix, Hoechst Pharmaceuticals, Somerville, New Jersey, USA) in 0.9% NaCl was administered during the experimental period as 0.2 mg/kg prime (0.07 ml) over a two minute interval, followed by a continuous i.v. infusion at the rate of 0.3 to 0.4 mg/kg/hr (0.58 ml/hr). This dosage was selected to reproduce the increase in urine flow rate observed during ANP infusion in the previous group and thus served as control for increases in tubule hydraulic pressure observed during diuresis [13].

In a single rat from each of these groups it was possible to position the pressure pipette within a descending vas rectum in a secure fashion such that it could be kept in place for several minutes during initiation of the priming dose of ANP or furosemide, thereby providing a continuous recording of the acute effects of these agents on vasa recta hydraulic pressure.

Group 3 (N = 7)

Rats in this group followed an identical protocol as those in Group 1, except that the plasma infusion rate was not increased until four to five minutes after the ANP priming dose had been given and the sustaining infusion had begun. This protocol was found in preliminary studies to be associated with unchanged whole kidney GFR and a modest increase in urinary sodium excretion ($U_{Na}V$).

Chemical analyses and calculations

Urine sodium concentration was measured by flame photometry (Model 443, Instrumentation Laboratory, Lexington, Massachusetts, USA). The concentrations of inulin and PAH in plasma and urine were determined, respectively, by a macroanthrone method [14] and by a colorimetric technique [15].

Postglomerular plasma oncotic pressure was calculated by standard formulas as previously described [16]. A value of 4.9 mg/dl for systemic plasma protein concentration was used for these calculations, a value obtained for the baseline period in rats of comparable age (N = 11), and assuming no change during the experimental period.

Statistical analysis

All results are expressed as means ± SEM. Statistical analyses were performed using one-way analysis of variance and paired t-tests, where appropriate. If the one-way analysis of variance determined the presence of differences between groups, a t-test using the Bonferroni modification for three preplanned comparisons was applied to determine significance [17]. Statistical significance was defined as $P < 0.05$.

Results

Baseline period

Mean values for the parameters studied in this initial period were similar in all three groups. Hydraulic pressures in descending vasa recta were slightly higher (9.1 ± 0.3, 8.4 ± 0.5, 9.6 ± 0.2 mm Hg; groups 1, 2, and 3, respectively) than in ascending vasa recta (7.8 ± 0.6, 7.8 ± 0.4, 8.1 ± 0.3 mm Hg; groups 1, 2, and 3, respectively), the axial gradient thus favoring forward blood flow. Hydraulic pressures similar to those of ascending vasa recta were found in loops of Henle (7.8 ± 0.4, 7.3 ± 0.3, 8.3 ± 0.5 mm Hg; groups 1, 2, and 3, respectively), while correspondingly lesser values were measured at the base (5.2 ± 0.3, 4.5 ± 0.2, 4.4 ± 0.1 mm Hg) and

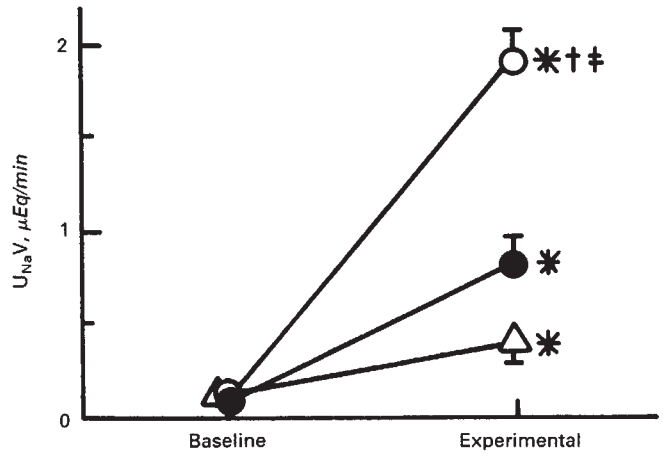

Fig. 1. *Urinary sodium excretion ($U_{Na}V$) in Groups 1 (○), 2 (●), and 3 (△) during baseline and experimental periods.* Values are expressed as means ± 1 SEM; * $P < 0.05$ vs. baseline, † $P < 0.05$ vs. group 2, ‡ $P < 0.05$ vs. group 3.

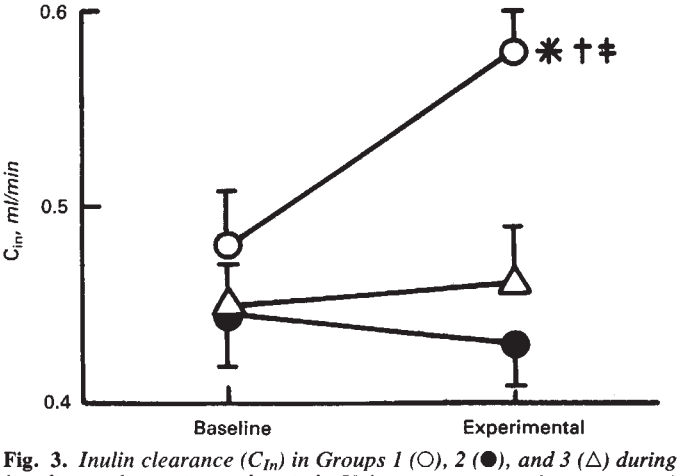

Fig. 3. *Inulin clearance (C_{In}) in Groups 1 (○), 2 (●), and 3 (△) during baseline and experimental periods.* Values are expressed as means ± 1 SEM; * $P < 0.05$ vs. baseline, † $P < 0.05$ vs. group 2, ‡ $P < 0.05$ vs. group 3.

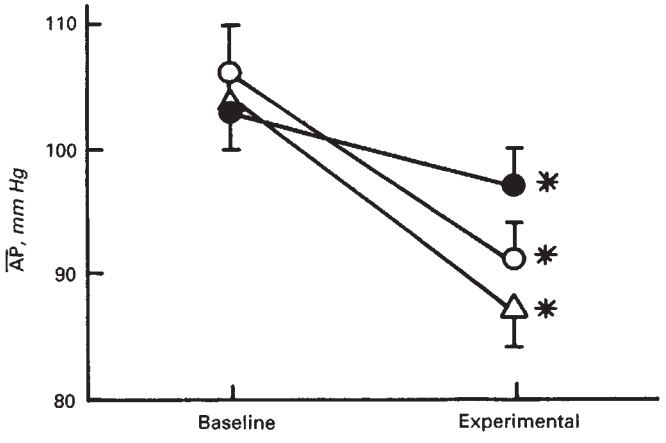

Fig. 2. *Mean systemic arterial pressure ($\overline{AP}$) in Groups 1 (○), 2 (●), and 3 (△) during baseline and experimental periods.* Values are expressed as means ± 1 SEM; * $P < 0.05$ vs. baseline.

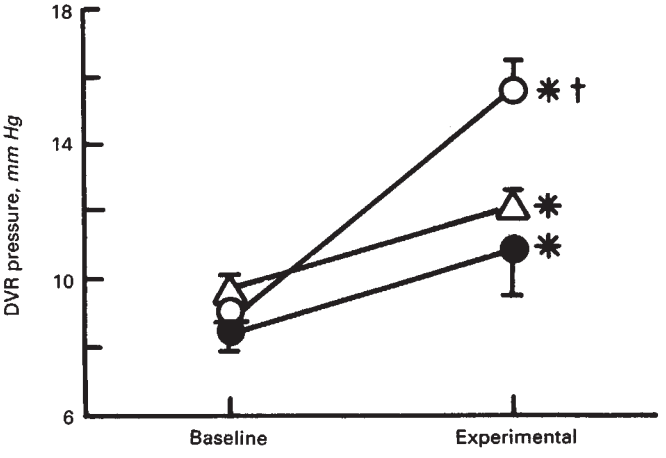

Fig. 4. *Hydraulic pressures in descending vasa recta (DVR) in Groups 1 (○), 2 (●), and 3 (△) during baseline and experimental periods.* Values are expressed as means ± 1 SEM; * $P < 0.05$ vs. baseline, † $P < 0.05$ vs. group 2.

mid-portion of the collecting duct (3.1 ± 0.5, 2.8 ± 0.3, 2.8 ± 0.2 mm Hg), with the axial pressure gradient in these structures again favoring forward flow of tubule fluid.

Experimental period

Group 1. As depicted in Figure 1, ANP infusion in this group of rats resulted in a more than 23-fold increase in $U_{Na}V$ in the setting of a 15 mm Hg reduction in $\overline{AP}$ (Fig. 2). This natriuretic response resulted not only from an elevation of V (from 1.4 ± 0.1 to 7.5 ± 0.8 μl/min, $P < 0.05$), but also from a marked rise in urine sodium concentration to substantially hypernatric levels (U_{Na}, from 54 ± 5 to 253 ± 6 mEq/liter, $P < 0.05$). Whole kidney inulin clearance, (C_{In}, Fig. 3), a measure of GFR, increased significantly, on average by 21% over baseline values, while effective renal plasma flow (RPF), estimated from the PAH clearance (C_{PAH}), was largely unaffected (1.56 ± 0.06 and 1.68 ± 0.07 ml/min, baseline and experimental periods, respectively). Hydraulic pressures in both vascular and tubule structures of the exposed renal papilla were significantly increased during infusion of ANP. Hydraulic pressures in tubule elements increased by approximately 2 mm Hg, on average in loops of Henle from 7.8 ± 0.4 to 10.1 ± 0.7 mm Hg, at the collecting duct base from 5.2 ± 0.3 to 7.1 ± 0.3 mm Hg, and at the mid-portion of the exposed collecting duct from 3.1 ± 0.5 to 5.2 ± 0.4 mm Hg. In contrast, the increment in vasa recta hydraulic pressures was much more marked, approximately threefold higher, than that observed in tubule elements, increasing on average in descending vasa recta from 9.1 ± 0.3 to 15.5 ± 1.0 mm Hg and in ascending vasa recta from 7.8 ± 0.6 to 14.3 ± 0.8 mm Hg (Figs. 4 and 5). Continuous recording in a descending vas rectum from the initiation of ANP administration showed that hydraulic pressure had increased immediately and progressively from 11 mm Hg to a maximum of 17 mm Hg within 90 seconds of beginning the ANP infusion. Time course determination of V in a single separate rat showed that $\dot{V}$ had approximately doubled within two minutes after initiation of the ANP prime (from 1.5 to 3.3 μl/min).

Group 2. Infusion of furosemide in Group 2 animals repro-

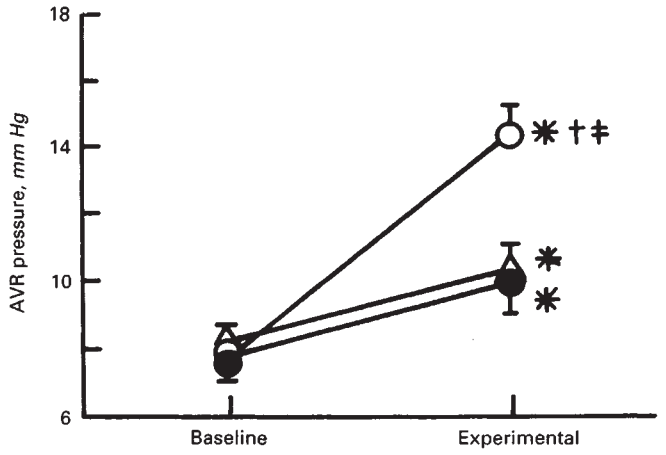

Fig. 5. *Hydraulic pressures in ascending vasa recta (AVR) in Groups 1 (○), 2 (●), and 3 (△) during baseline and experimental periods.* Values are expressed as means ± 1 SEM; * $P < 0.05$ vs. baseline, † $P < 0.05$ vs. group 2, ‡ $P < 0.05$ vs. group 3.

duced the high rate of $\dot{V}$ observed with ANP infusion in Group 1 (from 1.3 ± 0.2 to 8.3 ± 0.9 μl/min, $P < 0.05$). U_{Na} also increased significantly (from 51 ± 3 to 97 ± 5 mEq/liter), but this increase was of a significantly lesser magnitude than that achieved during ANP infusion in Group 1. This resulted in a less impressive increase in $U_{Na}V$ (Fig. 1). C_{In} (Fig. 3) remained relatively stable, in contrast to the rise observed in Group 1. $\overline{AP}$ also fell slightly but significantly by 6 mm Hg (Fig. 2). C_{PAH} remained essentially unchanged (1.47 ± 0.06 and 1.41 ± 0.05 ml/min, baseline and experimental period, respectively).

During infusion of furosemide, hydraulic pressures in tubule elements were increased significantly as in Group 1 by approximately 2 mm Hg, on average in loops of Henle from 7.3 ± 0.3 to 9.2 ± 0.4 mm Hg, at the collecting duct base from 4.5 ± 0.2 to 6.3 ± 0.4 mm Hg, and at the mid-portion of the collecting duct from 2.8 ± 0.3 to 4.4 ± 0.3 mm Hg. In contrast to Group 1 animals, however, the increment in vasa recta hydraulic pressures during furosemide administration (Figs. 4 and 5) was no greater in magnitude than that observed in tubule elements, increasing on average also by approximately 2 mm Hg in descending vasa recta (from 8.4 ± 0.5 to 10.8 ± 1.3 mm Hg) and in ascending vasa recta (from 7.8 ± 0.4 to 10.0 ± 1.0 mm Hg). Likewise, continuous monitoring of hydraulic pressure in a descending vas rectum during initiation and continued administration of furosemide revealed an increase from 8.5 to only 9.5 mm Hg within the initial two minutes of study, in contrast to the prompt and more marked rise in vascular hydraulic pressure observed upon infusion of ANP in Group 1.

Group 3. Infusion of ANP at an equivalent dose as in Group 1, but without commencing replacement of plasma volume losses until the diuresis was well established, produced a markedly blunted natriuretic response (Fig. 1), approximately 10% of the increment observed in Group 1. The increase in $\dot{V}$ (from 2.2 ± 0.4 to 3.7 ± 0.5 μl/min, $P < 0.05$) was significantly less than that which ocurred in both Groups 1 and 2. The rise in U_{Na} (from 56 ± 6 to 107 ± 17, $P < 0.05$) was also of substantially lesser magnitude than in Group 1, and resembled the increment seen during furosemide infusion. $\overline{AP}$ fell significantly, as occurred in the other two groups, but by a numerically greater amount of 17 mm Hg (Fig. 2). There was near-constancy of C_{In} during ANP infusion in Group 3 (Fig. 3), in contrast to the rise in C_{In} in Group 1. Also in contrast to the stable renal plasma flow rate in Groups 1 and 2, there was a reduction in C_{PAH} (from 1.36 ± 0.06 to 1.2 ± 0.06, $P < 0.05$).

The pattern of changes induced by ANP in hydraulic pressures in vasa recta were also different in rats in which plasma volume was maintained as compared to rats in which plasma volume replacement was delayed. Instead of the marked increases observed in Group 1 rats, Group 3 rats exhibited small increments in both descending and ascending vasa recta (Figs. 4 and 5), a response indistinguishable from that evoked by furosemide. The slight increase observed in tubule hydraulic pressures of Group 3 animals was similar to that ocurring in the previous two groups. Average pressures in the loop of Henle rose from 8.3 ± 0.5 to 9.5 ± 0.5 mm Hg, at the base of the collecting duct from 4.4 ± 0.1 to 5.7 ± 0.2 mm Hg, and in the mid-portion of the collecting duct from 2.8 ± 0.2 to 4.4 ± 0.2 mm Hg.

The calculated postglomerular plasma oncotic pressure changes between baseline and experimental periods were as follows: Group 1: +2.95 mm Hg, Group 2: −0.57 mm Hg, and Group 3: +3.65 mm Hg.

Discussion

Alterations in medullary hemodynamics have been considered to play a role in the regulation of renal sodium and water excretion [18]. The ability of renal vasodilators, such as acetylcholine and bradykinin, to cause natriuresis and diuresis has been correlated, in part, with medullary hemodynamic events. These include a shift in distribution of intrarenal blood flow toward inner cortex [19] and an increase in papillary plasma flow rate [20]. However, the precise interplay and relative importance of these factors in the control of intravascular volume have been difficult to define.

ANP has potent natriuretic and diuretic properties, acting as a preferential renal vasodilator [21, 22]. Its effect on the intrarenal vasculature is similar to that of previously studied renal vasodilators. Infusion of atrial peptides was associated with a shift in the distribution of renal blood flow from outer to juxtamedullary cortex and an increase in postglomerular plasma flow rate, as determined by microsphere and papillary ^{125}I-albumin uptake techniques [3, 6]. Papillary blood flow has also been shown to increase, as measured with a laser-Doppler flowmeter, within two minutes of infusion of synthetic ANP [10]. Vasa recta blood flow was augmented when measured by fluorescence videomicroscopy 45 minutes after initiation of ANP infusion [11]. These findings, taken together with the observed inhibition by ANP of collecting duct sodium reabsorption in vivo [7], point to inner medullary hemodynamic events as important determinants of the ANP-induced natriuresis and diuresis.

The mechanism by which vasodilators increase renal sodium and water excretion has been attributed, in part, to alterations in peritubular capillary Starling forces [23], such that reductions in preglomerular vasomotor tone allows transmission of a greater fraction of systemic pressure to glomerular and postglomerular peritubular capillaries. In concert with this proposed mechanism, increasing renal perfusion pressure during concomittant ANP infusion markedly potentiated the attendant

natriuresis and diuresis [16]. In contrast, raising the postglomerular vascular oncotic pressure effectively prevented the ANP-induced natriuresis and diuresis [16]. These observations indicate that peritubular capillary Starling forces can be important modulators of the ANP-induced natriuretic and diuretic effects.

Therefore, in view of the aforementioned effects of ANP on inner medullary hemodynamics, we sought to determine the hydraulic pressures in inner medullary structures in response to ANP and to compare them with the actions of a direct epithelial sodium transport inhibitor, furosemide, and to ANP administration in a setting which resulted in a blunted natriuresis.

Infusion of ANP in euvolemic Group 1 rats resulted in marked natriuresis and diuresis. Hydraulic pressures in tubule elements of the exposed renal papilla increased by approximately 2 mm Hg, an increment which closely matched the rise in proximal tubule hydraulic pressure observed previously in superficial cortex [21]. The rise in vasa recta hydraulic pressures during infusion of ANP in this group was much more marked, however, averaging approximately threefold higher than that observed in tubule elements. The presence of this pressure differential between the vascular and tubule elements indicates that this effect was not secondary to increases in loop transport or tubule fluid flow rates. Of particular note, this rise in papillary vascular pressures also exceeded the magnitude of increase in efferent arteriolar hydraulic pressure found in superficial cortex, which averaged only 2 mm Hg [21], thereby providing direct evidence for a preferential vasodilatory response to ANP infusion in medullary microvasculature.

In contrast to the effects of ANP and other potent vasodilators, studies of intrarenal blood flow distribution during furosemide infusion have revealed increases in flow only to midcortical areas, without enhancement in the juxtamedullary region [24]. Indeed, postglomerular plasma flow rate in the juxtamedullary region, as determined by papillary ^{125}I-albumin uptake in vivo, is reduced during furosemide infusion [25]. It is therefore not surprising that, in the present studies, furosemide infusion failed to preferentially augment vasa recta hydraulic pressures in Group 2 animals. In contrast to the pressure differential occurring between the vascular and tubule elements during ANP infusion in Group 1, administration of furosemide led to an approximately equal increment in hydraulic pressures (~2 mm Hg) in all medullary vascular and tubule structures. This small increment in vascular hydraulic pressures may have been due simply to a furosemide-induced increase in tubule pressures, in a manner analogous to that observed in superficial proximal tubules and adjacent peritubular capillaries following graded elevations of ureteral pressure or during mannitol diuresis [13]. Indeed, administration of furosemide has been shown to elevate hydraulic pressures in superficial proximal tubules and peritubular capillaries even when glomerular capillary hydraulic pressure remained stable and single nephron GFR declined [26]. Although the possibility of a vascular effect of furosemide independent of this agent's ability to augment tubule fluid flow rates cannot be excluded in the present study, the data serve to reinforce the concept that such an independent vascular effect must occur for ANP when its full hemodynamic effects are manifested.

Pre-renal arterial clamping has been demonstrated to blunt or abolish the ANP-induced natriuresis [1, 4, 9], an effect which has been ascribed to impeding the rise in GFR [1]. However, lowering renal perfusion pressure not only abolishes the rise in GFR but would also interfere with the occurrence of other hemodynamic events and, by itself, results in a lowering in renal sodium excretion [4, 9]. In the present study, failure to restore plasma volume during ANP administration in Group 3 rats was associated with unchanged GFR and a fall in RPF, a different whole kidney hemodynamic response than in Group 1. The natriuresis was also markedly attenuated (Fig. 1), consisting of approximately 10% of the increment in $U_{Na}V$ observed in Group 1 animals. In concert with the diminished natriuresis, the preferential increase in vasa recta hydraulic pressures also failed to occur. Instead, the hydraulic pressure increase in vasa recta was of the same magnitude as the rise in tubule hydraulic pressure, as occurred with furosemide infusion. The diminished, although significant, natriuretic response by Group 3 rats indicates that ANP possesses natriuretic actions independent of its effects on inner medullary and glomerular hemodynamics. This blunted natriuresis was of the same magnitude as that obtained when pre-renal arterial constriction was used to lower the GFR to pre-ANP infusion levels [1] and may represent the contribution of direct inhibition of sodium transport by the inner medullary collecting duct [8] in the absence of increased delivery to this segment. Thus, the medullary hemodynamic effects of ANP represent one of several factors contributing to natriuresis and diuresis, as discussed below. The observed lessening of the natriuretic and vascular effects in Group 3 might be due to the fall in renal perfusion pressure below 90 mm Hg, the level used in previous studies to impede renal hemodynamic effects of ANP [1, 4, 9]. The results obtained in this group underscore the importance of maintenance of plasma volume for full expression of the hemodynamic, and therefore, natriuretic and diuretic effects.

It is reasonable to assume that the pressure differential occurring between vascular and tubule elements in the papilla during ANP infusion in Group 1 would serve to limit fluid removal from the local interstitium, but only insofar as these increments in vascular hydraulic pressure exceeded any offsetting increases in vasa recta oncotic pressure. Although direct measurements of vasa recta oncotic pressure during infusion of ANP are not currently available, the calculated efferent arteriolar plasma oncotic pressure increased by less than 3 mm Hg in Group 1 rats, a value similar to that obtained for the superficial cortical efferent arteriolar oncotic pressure change [21]. This value would therefore serve as the upper limit for postglomerular plasma oncotic pressure change during the euvolemic ANP infusion studies. However, if the filtration fraction (FF) in deep glomeruli during ANP infusion falls with respect to that in superficial glomeruli, as occurs with infusions of other renal vasodilatory agents [27], and during a state known to be associated with ANP release [28], volume expansion with saline solutions [20, 30], there may be an even smaller, if any, increase in oncotic pressure within vasa recta. In addition, the preferential increase in blood flow to the deep nephron vasculature induced by ANP in the face of constant whole kidney plasma flow [4, 10, 11], suggests that indeed deep glomeruli may have a lower FF than superficial glomeruli during ANP infusion. It therefore appears unlikely that alterations in vasa recta oncotic pressure could offset the hydraulic pressure elevations observed in Group 1 rats. Moreover, since vasa recta are

postglomerular capillaries, this unopposed increase in hydraulic pressure is likely representative of the entire postglomerular circulation surrounding juxtamedullary nephrons, and could therefore limit fluid reabsorption at other sites in this nephron population as well. These events could explain the finding of increased fractional excretion of lithium during ANP infusion in intact animals [5, 6], despite the lack of inhibitory effect of this peptide on absolute sodium reabsorption by superficial proximal tubules in vivo [1] or by proximal straight or convoluted tubules from either superficial or juxtamedullary cortex in vitro [31]. Indeed, sodium delivery to the end-descending limb of juxtamedullary nephrons was increased by ANP infusion despite constant juxtamedullary single nephron GFR [32].

Videomicroscopic studies of the exposed renal papilla in vivo have demonstrated significant increases in vasa recta blood flow in response to infusion of ANP but revealed that such increments may lag behind the onset of natriuresis and diuresis, suggesting that the initial natriuretic effect of ANP resulted from actions other than the increase in blood flow [11]. This study showed a doubling of $U_{Na}V$ at two minutes after infusion of ANP and had increased further to fourfold over baseline values when measured at 45 minutes, at a time when vasa recta blood flows were significantly increased [11]. Thus, natriuresis was markedly enhanced at a time when vasa recta hydraulic pressure would have been expected to be highest. Moreover, as soon as two minutes following intravenous infusion of ANP, a significant rise in papillary blood flow has been demonstrated by other investigators in rats with exposed renal papilla using a laser-Doppler flowmeter technique [10]. The hydraulic pressure elevations in descending and ascending vasa recta in the present study were documented at 10 to 30 minutes after initiation of the ANP infusion, at a time when peak natriuresis had already been achieved. However, hydraulic pressure within a descending vas rectum in the present study, when measured continuously by an indwelling micropipette, was found to increase by several mm Hg within 90 seconds of initiating the systemic ANP infusion, at a time which appeared to coincide with the onset of diuresis. This indicates that changes in medullary hemodynamics may also be an important factor during the initial, as well as the maintenance, phases of the natriuretic response.

The findings in the present study offer insight not only into the mechanisms contributing to the natriuresis and diuresis induced by ANP but also concerning the markedly hypernatric character of the final urine. As shown in this study and previously, infusion of ANP augments GFR [1, 2, 16, 21], which not only enhances delivery of tubule fluid and solutes to more distal nephron segments [2], but also preserves salt transport in the medullary thick ascending limb of Henle [8], the latter promoting maintenance of a medullary interstitium more rich in sodium than is observed with administration of loop-active agents, such as furosemide [33]. ANP infusion also results in an elevation of vasa recta hydraulic pressure, almost certainly in excess of increments in oncotic pressure, thereby rendering local Starling forces less favorable for capillary fluid reabsorption, not only in the papilla, but in the entire postglomerular microcirculation surrounding juxtamedullary nephrons. Furthermore, the imbalance of hydraulic pressures between vascular and tubule elements could promote recycling of the relatively hypernatric papillary interstitial fluid favoring convective movement of sodium to adjacent collecting ducts. Although the medullary collecting duct of the golden hamster under antidiuretic conditions has been shown to have a high electrical resistance [34], the permeability of the inner medullary collecting duct during diuretic or volume expanded conditions is unknown. Indeed, preliminary in vitro studies have suggested that ANP may actually facilitate secretion of sodium by the inner medullary collecting duct [35]. This movement of sodium and water into the collecting duct lumen could occur through paracellular channels formed by permeable tight junctions known to be present in inner medullary collecting duct epithelia [36]. This possible recycling action by ANP, together with direct inhibition of active sodium transport in inner medullary collecting duct cells, would thereby confirm in vivo indications that ANP alters sodium transport in these terminal nephron segments [7]. Thus, the combination of enhanced distal sodium delivery through both an increase in GFR and unfavorable balance of vasa recta Starling forces, possible recycling of sodium from relatively hypernatric papillary interstitium to collecting duct lumen, and direct effects on inner medullary collecting duct sodium transport, all contribute to the large magnitude and hypernatric character of ANP-induced natriuresis and diuresis.

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